

REMARKS

Favorable reconsideration of this application, in light of the preceding amendments and following remarks, is respectfully requested.

Claims 1-6, 8-10 and 22 are pending in this application. Claims 1-6 and 8-9 have been withdrawn from consideration. Claims 8-10 are amended and claims 7 and 11-21 have been cancelled. Claim 22 has been added. Claims 1, 10 and 22 are the independent claims.

Applicants respectfully note that the present action does not indicate that the claim to foreign priority under 35 U.S.C. §119 has been acknowledged or that certified copies of all priority documents have been received by the U.S.P.T.O. Applicants respectfully request that the Examiner's next communication include an indication as to the claim to foreign priority under 35 U.S.C. §119 and an acknowledgement of receipt of the certified copies of all priority documents.

Applicants also respectfully note that the present action does not indicate that the drawings have been accepted by the Examiner. Applicants respectfully request that the Examiner's next communication include an indication as to the acceptability of the filed drawings or as to any perceived deficiencies so that the Applicants may have a full and fair opportunity to submit appropriate amendments and/or corrections to the drawings.

Example Embodiments of the Present Application

Independent claims 10 and 22 recite a homogeneously reacetylated chitosan having a molecular weight of not smaller than 200 kDa and a deacetylation degree of 30–60% for use in the preparation of a pseudo-thermosetting neutralized chitosan composition forming a phosphate-free transparent hydrogel at a temperature higher than 5°C. Independent claim 10 further recites a process for obtaining the homogeneously reacetylated chitosan defined as: filtrating a chitosan having a molecular weight of not smaller than 200 kDa and a deacetylation degree of 80 to 90 % dissolved in an acidic medium to eliminate insoluble particles; precipitating chitosan contained in the filtrated acidic solution to obtain chitosan free of insoluble particles; preparing a cooled acidic solution of the chitosan free of insoluble particles obtained at a temperature lower than 5°C to obtain a cooled acidic solution of chitosan free of insoluble particles; preparing a cooled acetic anhydride solution containing a predetermined amount of acetic anhydride in methanol at a temperature lower than 5°C; reacetylating chitosan by adding dropwise, under homogeneous conditions, the cooled acetic anhydride solution free of insoluble particles to the cooled solution of chitosan to provide a crude homogeneously reacetylated chitosan having a deacetylation degree of 30-60 %; treating said crude chitosan to eliminate salts produced during reacetylation and insoluble particles of chitosan to obtain a homogeneously reacetylated chitosan having a

deacetylation degree of 30-60 %. Example non-limiting embodiments of this feature are discussed, for example, in paragraphs [0073] and [0074] and Examples 3, 4, 8, 9, 10 and 11 of the instant specification.

As is illustrated in paragraphs [0073] and [0074] of the present application, homogeneity is an essential feature of the chitosan. A chitosan having a deacetylation degree of 49.0% (i.e. comprised between 30–60%) was reacetylated under non-homogeneous conditions (at room temperature, low stirring and acetic acid anhydride not being diluted in methanol before addition) does not form a pseudo-thermosetting neutralized chitosan composition forming a phosphate-free transparent hydrogel at a temperature higher than 5°C.

Rejections under 35 U.S.C. § 103

Domard and Granja in view of Varum, Baumann and Nettles

Claim 10 stands rejected under 35 U.S.C. § 103(a) as being obvious over WO 2002/078760 (U.S. Publication No. 2004/0171151) to Domard et al. (hereinafter “Domard”), as evidenced by *Industrial Research Ltd Catalog* (Jan. 14, 2009), and *Key Engineering Materials Vols.*, 254, 256 (2004) by Granja et al. (hereinafter “Granja”) and in view of WO 2003/011912 to Varum et al. (hereinafter “Varum”), *Carbohydrate Res* 1:43-57, (2001) by Baumann et al. (hereinafter “Baumann”) and *Tissue Engineering* 8:1009-1016, (2002) by

Nettles et al. (hereinafter "Nettles"). Applicants respectfully traverse this rejection for the reasons detailed below.

A. *Domard*

Domard is directed to a method for preparing a cartilaginous neo-tissue comprising contacting a chitosan hydrogel with a culture of chondrocyte cells in order to get adherence between the chitosan hydrogel and cultured chondrocyte cells and to lead to the development of a cartilaginous neo-tissue in contact with the surface of the chitosan hydrogel. Domard does not teach a process of homogeneously reacetylating a chitosan from a chitosan having a molecular weight of not smaller than 200 kDa and a deacetylation degree of 80 to 90% (i.e. an acetylation degree of 10-20%) as recited in claims 10 and 22. Rather, Domard teaches purification and later acetylation of a chitosan with an acetylation degree of <10% (e.g. of 5.2%) and is completely silent on the molecular weight of the said chitosan.

Domard teaches that for the use aimed at by Domard, i.e. as a cartilaginous neo tissue, a chitosan hydrogel with a deacetylation degree of 30 to 70%, notably 40-60% (i.e. an acetylation degree of 30-70%, notably 40-60%) is desirable. However, as disclosed in the present application as originally filed under Examples 3 and 4, a homogeneously reacetylated chitosan with a deacetylation degree higher than 60% (i.e. an acetylation degree lower than 40%) would not form a pseudo-thermosetting neutralized chitosan composition

forming a phosphate-free transparent hydrogel at a temperature higher than 5°C. From Domard, Applicants submit that one skilled in the art would not have any guidance to choose the source of chitosan as those chitosans are presented without preference (paragraph [0014] of Domard), but even further, one skilled in the art would be taught towards the use of a chitosan with an acetylation degree <10% subject to a reacetylation.

Furthermore, Domard does not teach a chitosan composition having pseudo-thermosetting properties suitable for the preparation of a transparent hydrogel as recited in claims 10 and 22. One skilled in the art of repairing cartilageneous lesions by grafting cartilageneous neo tissue would not be motivated to modify a hydrogel of Domard to obtain a transparent hydrogel of claims 10 and 22, notably suitable for ocular and topic preparation. Thus, Domard teaches a reacetylated chitosan which would not intrinsically have the properties of the claimed product (e.g., a homogeneously reacetylated chitosan having a molecular weight of not smaller than 200 kDa and a deacetylation degree of 30–60%) as recited in claims 10 and 22.

Therefore, starting from Domard, one skilled in the art would not have any guidance to choose the molecular weight of the chitosan (among known ranges of molecular weights for chitosans) for subjecting to a reacetylation, nor any guidance for selecting the deacetylation degree of the reacetylated chitosan as the molecular weight and deacetylation degree recited in claims 10 and 22,

when aiming at forming a pseudo-thermosetting chitosan composition forming a phosphate-free transparent hydrogel at a temperature higher than 5°C.

Regarding claim 10, Applicants further submit that Domard fails to give any specific guidance for performing the acetylation of chitosan and forming a hydrogel for use as a cartilaginous neo tissue, but rather provides only some generic steps in paragraph [0040], in particular, those steps which are suitable for ocular and topic preparation.

In particular, Domard does not teach a process of homogeneously reacetylating a chitosan from a chitosan having a molecular weight of not smaller than 200 kDa and a deacetylation degree of 80 to 90% (i.e. an acetylation degree of 10-20%) comprising preparing a cooled acidic solution of the chitosan free insoluble particles obtained at a temperature lower than 5°C, preparing [separately] a cooled anhydride solution containing a pre-determined amount of acetic anhydride in methanol at a temperature lower than 5°C, or reacetylating chitosan by adding dropwise, under homogeneous conditions, the cooled acetic anhydride solution to the cooled solution of chitosan to provide a crude homogeneous reacetylated chitosan having a deacetylation degree of 30-60% as recited in claim 10. The reacetylation process carried out at the low temperature described in claim 10 ensures a homogeneous reaction as disclosed in the specification, in particular, comparative examples 8 and 9. Applicants submit that Domard does not teach or suggest a reacetylation

process under homogeneous conditions and in particular at low temperature as recited in claim 10.

Finally, Domard does not teach a homogeneously reacetylated chitosan having a molecular weight of not smaller than 200 kDa and a deacetylation degree of 80 to 90% (i.e. an acetylation degree of 10-20%) made by treating said crude chitosan obtained to eliminate salts and insoluble particles as recited in claim 10. Further, Domard teaches a reacetylation process of chitosan in the presence of 1,2 propanediol, whereas the reacetylation process according to the invention does not use 1,2 propanediol.

Therefore, from Domard, one skilled in the art would not have been motivated to choose the acetylation conditions which would lead to a chitosan having the properties of the claimed product as recited in claim 10.

B. *Varum*

Varum relates to a process for the preparation of chitosan via heterogeneous deacetylation of chitin, rather than reacetylation of chitosan under homogeneous conditions as recited in claims 10 and 22, and thus, teaches away from the present invention, e.g., teaches away from homogeneous deacetylation of chitin for cost reasons (see p. 5 of Varum) and proposes a method of deacetylation of chitin in heterogenous conditions (after extended swelling of chitin). In addition, the teaching of Varum relating to the effect on

the distribution of acetylated and deacetylated units along the chain of homogeneous versus heterogeneous conditions when deacetylating chitin is somewhat contradictory: “NMR-studies have thus shown that water soluble chitosans prepared both homogeneously and heterogeneously have a random distribution of acetylated and deacetylated units along the chain” (see p. 3). “In the case of the homogeneous reaction, the remaining N-acetyl groups are generally randomly located along the polymeric backbone of the chitosan product. In the case of the heterogeneous reaction, a small fraction of insoluble chitin-like material is most often present in the product together with an acid-soluble fraction with a near random distribution of acetyl groups along the polymeric backbones” (p 4).

Further, Varum is completely silent not only on the molecular weight of the chitosan obtained after deacetylating chitin but also on the homogeneous reaction conditions for performing deacetylation of chitin, and does not even discuss reacetylation of chitosan.

C. Industrial Research Catalog

The internet reference to the Industrial Research Ltd Catalog does not show any date that can attest when it was first available to the public, and therefore, should not be considered as prior art available to one skilled in the art at the filing date of the present application. Even if the catalog was

available at the filing date of the present application, Industrial Research only discloses that "chitosan prepared by deacetylating a squid pen derived from β -chitin is expected to have a molecular weight higher than chitosan derived from other sources". However, Applicants submit that one skilled in the art would not have any guidance to choose a specific molecular weight range of chitosan among all the molecular weights available for chitosans (at least from about 5 kDa to 2,000 kDa as recited in claims 10 and 22), including among the molecular weight ranges which were available for chitosans obtained by deacetylating a squid pen derived from β -chitin.

D. Baumann

Baumann relates to a structure activity relationship study on regioselective reactions for the introduction of O-sulfo, N-sulfo, N-acetyl and N-carboxymethyl groups on chitosan. Baumann discloses a commercially available chitosan of a molecular weight of 150 kDa and an acetylation degree of 0.28 (e.g. deacetylation degree of 72%) and an acetylated chitosan with an acetylation degree of 0.45 (e.g. deacetylation degree of 55%). Baumann further teaches lowering the molecular weight of a chitosan of 150 kDa and of its acetylation degree of 0.28 by hydrolysis into a chitosan of 29 kDa and an acetylation degree of 0.14 (e.g. deacetylation degree of 86%) to decrease toxicity and viscosity and unease regioselective reactions (p 44). Therefore, Bauman

teaches away from a homogeneously reacylated chitosan having a molecular weight of not smaller than 200 kDa and a deacetylation degree of 30–60% as recited in claims 10 and 22.

Baumann mentions very vaguely that “most reactions” are to be carried out in homogenous media for avoiding solubility problems but do not provide any hint as to whether this general statement applies to reacylation reactions in particular, nor which homogenous “media” it would be, nor provide any guidance for performing a homogeneous reacylation according to claim 10, nor any teaching about the physical properties of a N-acetylated chitosan.

E. *Granja*

Granja relates to the preparation of injectable chitosan-hydroxyapatite microspheres to promote localized bone regeneration. Granja discloses a squid chitosan having a viscosity average molecular weight of 2,480 kDa and a degree of acetylation of 0.30 (e.g. deacetylation degree of 70%). Further, Granja discloses the heterogeneous deacetylation of this chitosan into a chitosan having a degree of acetylation of 0.14 (e.g. deacetylation degree of 86%). Granja mentions the effect of chitosan deacetylation on the decrease of the size of the microspheres. However, Granja does not deal with reacylation of chitosan nor provide any hint about the choice of the chitosan (nor molecular weight or acetylation degree range). Even if Granja would teach the use of high

molecular weight chitosan, e.g. 2,480 kDa (which Applicants do not admit), Granja would also teach towards the use of a chitosan with a high deacetylation degree (e.g. at least of 70%). Further, one skilled in the art in the field of repairing cartilageneous lesions by grafting cartilageneous neo tissue or promoting localized bone regeneration would not have the motivation of modifying a hydrogel of Domard or the microspheres of Granja to obtain a transparent hydrogel as claimed in claims 10 and 22, notably suitable for ocular and topic preparation.

F. *Nettles*

Nettles relates to a study on the use of chitosan as a porous scaffold for cartilage tissue engineering. Nettles discloses the use of a 86% deacetylated chitosan having a molecular weight of 200 kDa for the formation of lyophilized microstructures, which, when rehydrated, are put in contact with a tissue culture in order to assess cell viability and attachment to the chitosan scaffold, but does not deal with reacetylation of chitosan. Nettles states that “the properties of porous chitosan matrices such as microstructure, crystalline and mechanical strength can be varied by altering chitosan concentration, freezing rate, molecular weight and percent of deacetylation of the starting material.” However, Applicants submit that no guidance is given to one skilled in the art about the role of these multiple parameters and the direction for at least one of

those parameters. At most, Nettles is an invitation to start a full research program to study the role of those different parameters in the formation of a porous scaffold for cartilage tissue engineering.

As disclosed in the present application as originally filed in Example 11, a commercially available chitosan with a deacetylation degree of 83.2% would not form a pseudo-thermosetting neutralized chitosan composition forming a phosphate-free transparent hydrogel at a temperature higher than 5°C.

In addition, one skilled in the art in the field of building porous scaffold for cell attachment in cartilage tissue engineering would not have the motivation of modifying the microstructures of Nettles to obtain a transparent hydrogel as recited in claims 10 and 22, notably suitable for ocular and topic preparation. Further, Nettles does not provide any motivation for the choice of the chitosan (nor molecular weight or acetylation degree range), nor provide any guidance for performing a homogeneous reacetylation according to claim 10, nor any teaching about the properties of a homogeneous reacetylated chitosan of claims 10 and 22. Further, Nettles teaches the use of a chitosan with a high deacetylation degree (e.g. of 86%).

G. Conclusion

In view of the above, Applicants respectfully submit that neither Domard, Industrial Research Catalog, Granja, Varum, Baumann, Nettles or the

combination thereof renders claims 10 and 22 obvious. In addition, as discussed above, the chitosans taught by the cited art would not intrinsically have the properties of the claimed product as recited in claims 10 and 22, and therefore, the product of claims 10 and 22 is patentably distinct from the cited art either alone or in combination with any of the cited art.

The Applicants, therefore, respectfully request that the rejection to Claim 10 under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

In view of the above remarks and amendments, the Applicants respectfully submit that each of the pending objections and rejections has been addressed and overcome, placing the present application in condition for allowance. A notice to that effect is respectfully requested. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to contact the undersigned.


Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Erin G. Hoffman, Reg. No. 57,752, at the telephone number of the undersigned below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 08-0750 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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